Reactions to honeybee stings: an allergic prospective

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Purpose of review
The purpose of this article is to provide a brief overview of the events involved in honeybee allergy and to concisely update the reader on progress toward knowledge of honeybee venom (HBV), strides in solving diagnostic difficulties, and advancements in improving safety and efficacy of HBV immunotherapy.

Recent findings
It is well known that honeybee allergy is unique in venom allergen and protein composition, diagnostic challenges, and immunotherapy safety and efficacy. Many new honeybee allergens have been recognized. Advances in testing, evaluation, and extract manipulation methods, many using recombinant technology, have allowed a greater ability to help with honeybee allergy diagnosis and resultant improvement in immunotherapy safety and evaluation of immunotherapy efficacy.

Summary
In an effort to address many honeybee allergy concerns, specific advances have been recently made. Some recently characterized honeybee allergens appear to be major contributors to honeybee allergy. In the setting of double-positivity, cross-reacting carbohydrate determinants and other cross-reacting components in HBV have made diagnosis of honeybee allergy challenging. Recombinant technology, including component-resolved diagnostics, and other evolving testing methods should help clarify double-positivity, if not now, in the very near future. Purified HBV and possibly depot formulations for immunotherapy appear to make it more well tolerated. Recombinant methods may help with evaluation of immunotherapy's safety and efficacy.

Keywords
component resolved diagnostics, cross-reacting carbohydrate determinant, honeybee allergy, recombinant venom allergen, venom immunotherapy

INTRODUCTION

To the practicing allergist, venom allergy is important because of the life-protecting implications of recognition and proper therapy. More than other flying Hymenoptera, honeybee allergy presents unique challenges. Diagnosis of true honeybee sensitization, especially in the face of double-positivity, and the efficacy and safety of honeybee venom immunotherapy (VIT) are often problematic and remain the focus of ongoing research. After reviewing honeybee sting reactions, this review summarizes recent findings in allergenic honeybee venom (HBV) components, progress to solve challenges in diagnosing honeybee sensitization and hence true allergy, and strides in making honeybee VIT more well tolerated and efficacious.

HONEYBEE STINGS

Reactions to honeybee stings range from small local reactions to large local reactions to anaphylaxis and even death. Honeybee sting reactions may even be toxic, particularly mass envenomations, and cause rhabdomyolysis, hemolysis, thrombocytopenia, acute renal failure, hepatitis, mental status changes, and cardiac arrest [1,2]. Though solely honeybee data are lacking, stings seem to occur with a prevalence of about 30–40% among the adult population with a systemic reaction rate of 0.5–2% [3–5]. Death rates for honeybee stings are not well established, but one study [6] in the United States, likely underreported,

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KEY POINTS

- Honeybee allergy presents unique challenges in that its venom is more complex, it is more often is the cause of double-positivity, and its venom immunotherapy is less well tolerated and less efficacious than other Hymenoptera.
- Three new HBV allergens appear to be significant contributors to honeybee allergy, one of which may be a new major allergen.
- Component-resolved diagnostics, using recombinant technology, may be useful in resolving double-positivity, though it is not ready to replace skin testing as the primary tool for evaluation.
- Tools for evaluating venom immunotherapy efficacy and safety have shown promise, and depot injection and purified extract injection are likely more well tolerated and equally effective compared with standard immunotherapy extracts.
- The basophil activation test may aid in both resolving double-positivity and in evaluating immunotherapy efficacy; however, it remains a difficult test to standardize and operate.

showed half of the 40 deaths per year were due to honeybees.

Eliciting stings

We tend to excite honeybees when we disturb their foraging behavior, typically through stepping on them or agitating them through abrupt, proximal movements (such as swatting or gardening) [7]. Fortunately, honeybees are docile, so stings are rare relative to encounters. The aggressiveness of honeybees depends on a number of factors to include air temperature, humidity, time of day, specific season, physical threat, honeybee species (Africanized honeybee being more aggressive), and perhaps odor [8–10]. Contrary to common conception, 'looking like a flower' shows little risk for a honeybee sting [7].

Honeybee sting mechanics

The honeybee stinging apparatus and mechanics have been described earlier [11▪]. Unique to the honeybee are multibarbed lancets that are prominent and that facilitate autotomy, making a sting more likely from a honeybee when the stinger is found at the sting site. Approximately, 50–140 μg of HBV on average is reported from a sting, with volumes as high as 300 μg [12,13]. It is important to note that honeybee sting volumes can vary widely, as this may explain, at least in part, variable reactions during field stings and sting challenges.

Various routes of sensitization and reaction

Various routes of HBV sensitization and reaction have been reported recently, aside from the obvious sting and previously reported royal jelly, propolis, and other components of the hive [14,15]. A child experienced a systemic reaction after secondary mucocutaneous exposure to HBV on a glass from which he drank, which his father, a hobbyist beekeeper, had handled [16]. In a recent study [17] from Korea, it was noted that the only source of sensitization for 10% of their honeybee-allergic patients was from acupuncture with HBV extract, a common practice for pain relief in Oriental medicine. The important lesson is that sensitization and reaction can come through nonconventional means, so detailed history and open-mindedness continue to be paramount.

HONEYBEE ALLERGENIC COMPONENTS

Honeybee-predominant concerns of VIT efficacy/safety and double-positivity drive continued intense interest in further characterizing HBV components. Table 1 [18–25,26▪▪,27▪] lists the current, known HBV allergens. Phospholipase A_2 (Api m 1), a glycoprotein, is the most potent and allergenic protein. Aside from eliciting a response through direct stimulation by cross-linking IgE on mast cells and basophils, it also increases leukotriene production through the hydrolysis of phospholipids [28].

Hyaluronidase (Api m 2) is traditionally thought to be the second most allergenic substance and is found to have about 50% sequence identity with its Vespid counterpart [29]. Recent findings regarding the antigenicity of other HBV allergens, discussed below, seem to challenge the role of hyaluronidase as a major honeybee allergen, especially in light of a recent study [30] showing that cross-reacting carbohydrate determinates (CCDs) account for most of the specific IgE in Vespid hyaluronidase and that much of the cross-reactivity with the honeybee hyaluronidase is likely because of these CCDs.

Melittin (Api m 4) is the largest contributor to HBV composition. It is responsible for much of the pain and inflammation during the sting, likely via cell lysis (red blood cells, myocytes, leukocytes, mast cells, among others) [31]. Although it seems to be a relatively weak allergen, it remains a very important HBV protein due to its abundance, ability to potentiate other venom proteins, and toxicity potential. Api m 3, 5, 6, and 7 show a substantial preponderance for causing sensitization in individuals, with

Table 1. Honeybee venom allergenic components (www.allergen.org), ? = unknown

	Allergen	Size (kD)	Venom (%)	IgE positive (%)	Allergenicity	Glycosylation	Cross-reactivity
Api m 1	Phospholipase A_2	16	7–15	95 [18]	Very high	Yes	Low
Api m 2	Hyaluronidase	39	1–3	50 [19,20]	Weak to moderate	Yes	High
Api m 3	Acid phosphatase	43	1	37 [21]	Weak	Yes	Moderate
Api m 4	Melittin	3	35–50	28–50 [22]	Weak	No	Low
Api m 5	Dipeptidylpeptidase IV	100	1	60 [23]	?	Yes	Moderate
Api m 6	Cysteine-rich trypsin inhibitor	8	1–2	42 [24]	Weak	No	?
Api m 7	CUB serine protease	39	<1	80 [25]	?	Yes	?
Api m 8	Carboxyesterase	70	<1	?	?	?	?
Api m 9	Serine carboxyesterase	60	?	?	?	?	?
Api m 10	Icarapin	50–55	?	50 [26▪▪]	Moderate to high	Yes	Low
Api m 11	MRJP	–	–	–	–	–	–
11.0101	MRJP 8	50–60	?	15 [27▪]	Weak	Yes	?
11.0201	MRJP 9	50–60	?	34 [27▪]	Weak	Yes	?
Api m 12	Vitellogenin	200	?	?	?	?	?

CUB, Complement subcomponents Clr/Cls, Uegf, and Bone morphogenic protein-1; MRJP, major royal jelly protein.

reported 30–80% of honeybee-allergic patients being sensitized; however, it is yet unclear in these how much of the sensitivity is owing to CCDs or what is their clinical significance [23–25,32]. Further studies are needed for these potentially allergenically important components.

A fairly recently discovered HBV allergen, icarapin (Api m 10), a carbohydrate-rich protein of unknown function, seems to show genuine and perhaps honeybee-specific allergenicity, exhibiting 50% sensitization among honeybee-allergic patients, regardless of glycosylation (CCDs), which more strongly supports a true sensitivity [26▪▪]. This protein, though, seems to be particularly labile, which has made it elusive to analyze, so its true contribution to HBV composition remains unknown. Because of its labile nature, therapeutic immunotherapy may be affected by lack of this substance, as discussed later.

Major royal jelly proteins (Api m 11) have also shown promise as new potential allergens within the HBV. A recent study [27▪] showed that more than half of patients showed specific IgE to two of these proteins. However, CCDs seemed to contribute a substantial amount to these sensitivities. Specific IgE to the nonglycosylated forms, though, demonstrate that the allergic potential of these proteins should not be ignored.

Another new allergen, vitellogenin (Api m 12), a glycolipoprotein involved in fat storage and deposition, is a protein found in many animals (most notably fish) and their yolks, and it is even an allergen in Vespid venom (Ves v 6) [33]. Because of its abundance in many species, this allergen may also account for some degree of cross-reactivity between Hymenoptera venom [34]. It is likely similar to CCDs, being responsible for clinically nonimportant sensitization.

DIFFICULTIES WITH HONEYBEE ALLERGY DIAGNOSIS

The conundrum every allergist faces with venom allergy, particularly with HBV allergy, is the double-positive or multipositive patient, especially with a history of a single sting or multiple stings to a single, known insect. Double-positivity may be because of either true double-positive allergy or cross-reacting substances such as shared amino-acid sequences between venoms or because of CCDs. Methods to clarify these difficult patients have been recently researched and hotly debated.

Current diagnostic standard

According to current guidelines in the United States, evaluation of a patient with a concerning history begins with skin testing using extracted honeybee venom standardized to Api m 1 [35]. Intradermal doses start typically at a concentration of 0.001 μg/ml and progress to 1 μg/ml. These concentrations produce sensitivity, based on strong clinical history, of more than 95% [36,37]. The specificity, however, of intradermal testing may be considerably lower (70–75%), likely because of the myriad of disruptive substances in HBV.

Current serologic methods

Current methods for serologic testing, using immunoassay capture testing (CAP), a solid-phase immunoassay, to determine honeybee venom sensitization use whole venom, again, standardized to Api m 1. The sensitivity and specificity of current methods, based on strong clinical history, is about 93% and 84%, at best, respectively [38]. Though most feel serologic testing is second-line, some allergists may consider using CAP-specific IgE even as their primary test, which is not unreasonable.

Recombinant allergen serologic testing

As it continues to advance, serologic testing seems to be the way of the future for HBV allergy diagnostic testing for several reasons: convenience to the patient (less time, less pain, ability to stay on antihistamine medication), safety (no chance of reaction compared with at least a minimal chance to react to the skin testing), and soon a more refined detection of venom allergen sensitivity, especially with the advent of recombinant allergen technology utilizing component-resolved diagnostics (CRD). Several studies [39–41,42▪▪], using recombinant Api m 1 (rApi m 1) as a surrogate for honeybee allergy detection via serologic CAP, show a sensitivity and specificity, based on clinical history and skin testing, of 60–80% and 100%, respectively. By comparison, Vespid venom components (using both rVes v 1 and rVes v 5) showed a sensitivity of about 94% [43]. Several of these studies concluded as well that many variables, such as geographical, methodological, material, and population differences, may have accounted for the wide range of sensitivities.

Though much correspondence debated the usefulness of rApi m 1 in the diagnosis of HBV allergy, all universally agreed that the sensitivity could be dramatically improved by adding several other recombinant HBV allergens; however, this has not been specifically well studied yet. Although CRD may be good enough one day to be the primary diagnostic tool, its reasonable sensitivity and excellent specificity may make it good enough now as a limited tool to help distinguish double-positivity, as has been proposed [42▪▪]. Unfortunately, CRD for HBV is not currently available for use in the United States, though rApi m 1 is available for commercial use in Europe. As it develops, cost, reproducible and reliable results among laboratories, and access of the testing to allergists are all concerns and potential drawbacks to address.

Role of cross-reacting carbohydrate determinants

CCDs remain at the forefront of discussion and were initially confirmed by one group to elicit more double-positivity in honeybee-allergic patients [44]. Recently, another group confirmed this and showed that the vast majority (81%) of double-positive, anti-CCD IgE-positive patients were positive to rApi m 1 versus 64% to rVes v 5 [40]. By looking at their interesting data, it can also be concluded that roughly 25% of the double-positive patients can be attributed to CCDs, three-eighths to true double-positivity to rApi m 1 and rVes v 5, and three-eighths it seems to other factors, which may be specific IgE to other venom components (e.g. Api m 3, Api m 10, among others), cross-reacting components (e.g. Api m 2 and Ves v 2), or yet unrecognized allergens. It may have been of interest to stratify the double-positive/CCD data according to specific insect allergy. This may offer further insight into what role the anti-CCD IgE may truly play in honeybee allergy, in particular.

Use of purified honeybee allergens in diagnosis

Purified, natural Api m 1 (nApi m 1) has been studied recently and postulated to help resolve double-positivity. One group concluded that although nApi m 1 was more sensitive, rApi m 1 seemed to catch more truly sensitized individuals, owing to its absence of CCDs [41]. This conclusion was countered by data from another group showing that nApi m 1 was more positive than rApi m 1 in patients with more severe reactions, suggesting that using solely rApi m 1 would miss some critical patients [45]. What cannot be concluded from either of these studies is whether there are simply other substances that have greater clinical significance, such as specific IgE to other HBV allergens, and that the greater sensitivity of nApi m 1 is simply getting 'lucky' by inadvertently being positive because of anti-CCD IgE that is lacking on rApi m 1. What can be concluded is that we are far from understanding double-positive patients, yet a little closer at the same time, and perhaps by continuing studies to include more recombinant HBV components, we will gain further insight.

Basophil activation testing and other considerations

The basophil activation test (BAT) continues to be revisited as a means to distinguish double-positivity. One group recently showed that BAT may compare well against recombinant allergen-based IgE to honeybee and Vespid when evaluating the double-positive patient and may even prove to be a 'next-step' test to distinguish those who are missed by standard methods and/or CRD [46]. The BAT

procedure, however, is wrought with challenges such as procedures varying among laboratories, end points varying among studies reported (i.e. 15% basophil activation versus 50% or greater as a cut off), and being technically challenging and time consuming for the laboratory technician. Specific IgE-inhibition testing can be useful in distinguishing double-positivity as well; however, like BAT, and perhaps more so, it remains technically difficult and time-intensive and is typically only performed in the research setting.

SAFETY AND EFFICACY OF HONEYBEE IMMUNOTHERAPY

Immunotherapy remains the main treatment for preventing future sting reactions by providing long-lasting protection [35]. Compared with Vespid VIT, honeybee VIT is less effective and appears to elicit more systemic reactions, making it less well tolerated as well. For these reasons, studies of late are focused on these areas. A recent, comprehensive review specifically on honeybee VIT discussed many of these findings among other important considerations and is worth the reader's review [47▪]. The mechanism of VIT to honeybee and Vespid was also recently thoroughly reviewed [48].

Evaluating venom immunotherapy efficacy

No recent study has evaluated methods to exclusively improve the efficacy of honeybee VIT. However, some studies, discussed below, designed mainly to improve safety, also demonstrated non-inferior effective treatment. Because of the poorer efficacy of honeybee VIT, one study [49▪] sought to find a more consistent way to evaluate for VIT effectiveness in a patient by using a microsyringe injection of natural HBV as a surrogate for a real honeybee sting. The syringe method was at least as effective as a natural honeybee sting. This concept of using a surrogate for a true honeybee sting is new, and may show promise for standardizing a type of 'sting challenge' by syringe with known components, thus eliminating the inconsistency of honeybee sting challenges. This may hold diagnostic implications as well.

Another concept, introduced earlier in this article, considers that honeybee VIT may be less efficacious because of the absence of specific HBV allergens, perhaps lost during processing of the venom extract. Api m 10 was not found in any measureable concentration in therapeutic HBV preparations, though it appears to be a significant allergen [26▪▪]. Though there seem to be few data, lack of specific allergens in HBV extract may explain, at least in part, lower honeybee VIT efficacy, and future research should be strongly considered in this area.

Overall venom immunotherapy safety

A recently published Cochrane review affirmed, through thorough search and strict inclusion criteria, that honeybee VIT is less well tolerated with a systemic reaction rate of 14.2 versus 2.8% for Vespid VIT [50]. Another all-inclusive systematic review of the safety of VIT showed a higher systemic reaction rate for the honeybee VIT of about 25% [51▪]. Both of these reviews confirm well established data regarding a less-than-optimal safety profile for honeybee VIT.

Safety of aqueous versus depot extracts

In an effort to improve honeybee VIT safety, the review group from above attempted to separate aqueous extracts from depot extracts. Although, there was a difference in systemic reaction rates between depot and aqueous injections for honeybee VIT, the authors pointed out that this difference may be attributed to rush and cluster protocols for the aqueous and depot injections, respectively [51▪]. In another recent study [52▪▪], the authors felt, through cutting-edge spectrometry techniques, that tissue injected with aqueous extract showed greater amounts of dopamine, histidine, norepinephrine, and leukotrienes, suggesting an increased risk for adverse reactions. They also showed that HBV allergens remained in tissue longer following depot injections. Little can be concluded from these studies clinically, but certainly this testing confirms the prolongation of depot preparations and suggests reasons for their improved safety. This study also opens the door for further tissue pharmacokinetic studies that can be of use for the design of future effective HBV extracts and evaluation of specific HBV components.

Safety of purified versus nonpurified extracts

Purified HBV is devoid of the low-molecular-weight proteins that may be responsible, at least in part, for some of the adverse events in honeybee allergy and VIT. One group prospectively found a significant reduction in the systemic reaction rate of 2.5 versus 27.5% in the purified versus nonpurified groups, respectively [53▪]. Another group also showed similar low systemic reaction rates [54]. Interestingly, this latter group found that regardless of build-up schedule, the systemic reaction rates varied

little. Although both studies had some weaknesses (low numbers, nonblinded, nonrandomized), these are the first comparative, prospective studies evaluating what appears to be a well tolerated and likely equally effective alternative to the current honeybee VIT standard.

Tryptase and honeybee venom immunotherapy

For those on VIT, use of HBV is an independent risk factor for a systemic reaction, but interestingly, an elevated tryptase did not seem to put those on honeybee VIT at any higher risk, unlike for those on Vespid VIT [55]. A more recent study [56] using decreasing BAT positivity as a measure of protection also demonstrated that honeybee VIT systemic reaction rate did not correlate with tryptase levels. For now, mild tryptase elevation does not seem to be a factor for honeybee allergic patients. Caution should be taken, however, to not misinterpret this to mean that those with mast cell disorders are at no higher risk, as this group represents a separate entity entirely, recently discussed thoroughly elsewhere [57].

Basophil activation testing

One group has recently shown in two separate studies [56,58] that decreasing BAT positivity seems to correlate with honeybee VIT efficacy in children. Though this may one day be more mainstream, as previously discussed, concerns regarding BAT still make this testing method less useful to the practicing allergist, but certainly a considered option.

CONCLUSION

Allergy to honeybees poses unique problems for the allergist. Their complex venom composition, lack of more thorough understanding of HBV allergens, and the enigma of the double-positive honeybee-allergic patients lead to difficulty in diagnosis and management. Fortunately, CRD, recombinant technology, and clever allergists appear to be leading us to a greater understanding of effective solutions.

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Conflicts of interest

There are no conflicts of interest.

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